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IR-Mapping of Protein and Substrate Distributions within Novozyme-435 Beads

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Introduction: Novozyme-435, which is Lipase B from *Candida antartica* (CALB) immobilized within a macro porous poly (methyl methacrylate) resin (Lewatit), has extraordinary polyester syntheses activity. [1] Critical parameters for the performance of an immobilized enzyme such as Novozyme-435 are the spatial distributions of the enzyme and substrate(s). [2] This paper reports a novel method where FTIR spectral signals were the basis for the direct quantitative mapping of CALB and model substrates within cut Novozyme-435 bead slices.

Methods and Materials: Novozyme-435 beads were provided by Novozymes (Denmark). Monodispersed poly(styrene) standard were purchased from Polymer Laboratories Ltd. Novozyme-435 beads were incubated in 50% monodispersed poly(styrene) toluene- d_8 solution at 70°C for 2h. Novozyme-435 beads were microtomed to provide 9-micron thickness slices for use of IR in the transmission mode. The FTIR microscope at the National Synchrotron Light Source (NSLS) was used to perform IR mapping of the enzyme and substrate distribution within Novozyme-435 bead.

Results: This paper reports a novel method where FTIR spectral signals were the basis for the direct quantitative mapping of CALB and model substrates within cut bead slices. This provided an important alternative to the use of fluorescent tags on proteins that can deactivate the protein as well as introduce artifacts. The FTIR microscope at the National Synchrotron Light Source (NSLS) provided images with 10-micron spatial resolution. The most distinctive spectral features for the protein were the strong amide I and II bands centered at approximately at 1650 and 1540 cm^{-1} , respectively. For the bead matrix, which is composed of poly (methyl methacrylate), a distinctive peak due to ester groups was located in the region 1770-1700 cm^{-1} . Based on these differences between protein and bead matrix in the mid-IR, the distribution of protein in the slices was followed by raster scanning the sample and acquiring IR spectra from small areas (10 micron diameter). The 3-D or 2-D contour plots of the absolute peak values of the amide I bands for each spectrum versus the x and y positions were constructed. From study of these plots it was concluded that CALB within Novozyme-435 beads is located only at the outer 100 microns of beads with an average diameter of 600 microns. It is likely that the restricted diffusion of CALB within bead pores may significantly influence the immobilization process. This would be most anticipated when the characteristic size of the enzyme is similar to the pore size of the support. In this case, the average pore size for matrix bead is about 15 nm and the effective diameter of CALB is approximately 6.5 nm. Thus, we believe that the flow of enzyme into the pore is reduced to very small values by previous enzyme attachment, even though the pore is not physically blocked to the extent that other molecules cannot enter the bead. This later point was illustrated by studies with model polystyrene standards with molecular weights up to 46 000 g/mol. IR-imaging showed that the polystyrene was distributed uniformly throughout the beads. This difference in substrate and enzyme locations allows us to now explore substrate diffusivity within the beads and correlate this to the observed kinetics of enzymatic polymerizations.

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